

WHAT IS CLAIMED IS:

- Sub  
A
1. A device or integrated system, comprising: a physical or logical array of reaction mixtures, each reaction mixture comprising one or more shuffled or mutagenized nucleic acids or one or more transcribed shuffled or transcribed mutagenized nucleic acids and one or more in vitro translation reagents.
  2. The device or integrated system of claim 1, further comprising a duplicate of the physical or logical array.
  3. The device or integrated system of claim 1, further comprising a bar-code based sample tracking module, which module comprises a bar code reader and a computer readable database comprising at least one entry for at least one array or at least one array member, which entry is corresponded to at least one bar code.
  4. The device or integrated system of claim 1, further a long term storage device comprising of one or more of: a refrigerator; an electrically powered cooling device; a device capable of maintaining a temperature of  $< 0^{\circ}\text{C}$ ; a freezer; a device which uses liquid nitrogen or liquid helium for cooling storing or freezing samples, a container comprising wet or dry ice, a constant temperature and/or constant humidity chamber or incubator; or an automated sample storage or retrieval unit.
  5. The device or integrated system of claim 4, further comprising one or more modules for moving arrays or array members into the long term storage device.
  6. The device or integrated system of claim 1, further comprising a copy array comprising a copy of each of a plurality of members of the one or more shuffled or mutagenized nucleic acids in a physically or logically accessible arrangement of the members.
  7. The device or integrated system of claim 1, wherein a plurality of the reaction mixtures further comprise one or more translation products or one or more

Sub  
A/

transcription products, or both one or more translation products and one or more transcription products.

8. The device or integrated system of claim 1, wherein the array of reaction mixtures comprises a solid phase, liquid phase or mixed phase array of one or more of: the one or more shuffled nucleic acids, the one or more transcribed shuffled nucleic acids, or the one or more in vitro translation reagents.

9. The device or integrated system of claim 1, wherein the one or more shuffled nucleic acids are homologous.

10. The device or integrated system of claim 1, wherein the one or more transcribed shuffled nucleic acid is an mRNA.

11. The device or integrated system of claim 1, wherein the one or more in vitro translation reagents comprise one or more of: a reticulocyte lysate, a rabbit reticulocyte lysate, a canine microsome translation mixture, a wheat germ in vitro translation (IVT) mixture, or an *E coli* lysate.

12. The device or integrated system of claim 1, further comprising one or more in vitro transcription reagents.

13. The device or system of claim 12, wherein the in vitro transcription reagents comprises one or more of: an *E. coli* lysate, an *E. coli* extract, an *E coli* s20 extract, a canine microsome system, a HeLa nuclear extract in vitro transcription component, an SP6/polymerase, a T3 polymerase or a T7 RNA polymerase

14. The device or integrated system of claim 1, further comprising a nucleic acid shuffling or mutagenesis module, which nucleic acid shuffling or mutagenesis module accepts input nucleic acids or character strings corresponding to input nucleic acids and manipulates the input nucleic acids or the character strings corresponding to input nucleic acids to produce output nucleic acids, which output nucleic acids comprise the one or more shuffled or mutagenized nucleic acids in the reaction mixture array.

15. The device or integrated system of claim 14, wherein the output nucleic acids comprise one or more sequence which controls transcription or translation.

16. The device or integrated system of claim 14, wherein the nucleic acid shuffling or mutagenesis module comprises a DNA shuffling module, which DNA module accepts input DNAs or character strings corresponding to input DNAs and manipulates the input DNAs or the character strings corresponding to input DNAs to produce output DNAs, which output DNAs comprise the one or more shuffled DNAs in the reaction mixture array.

17. The device or integrated system of claim 1, wherein the nucleic acid shuffling or mutagenesis module is preceded by a module which allows overlapping synthetic oligonucleotides to be first assembled into oligonucleotide multimers or functional open reading frames prior to entering the mutagenesis or shuffling module.

18. The device or integrated system of claim 14, wherein one or more module comprises or is operatively linked to a thermocycling device.

19. The device or integrated system of claim 14, wherein the nucleic acid shuffling or mutagenesis module comprises a mutagenesis module, which mutagenesis module mutagenizes the DNA.

20. The device or integrated system of claim 14, wherein the nucleic acid shuffling or mutagenesis module fragments the input nucleic acids to produce nucleic acid fragments, or wherein the input nucleic acids comprises cleaved or synthetic nucleic acid fragments.

21. The device or integrated system of claim 14, wherein the shuffling or mutagenesis module is mechanically, electronically, robotically or fluidically coupled to at least one other array operation module.

22. The device or integrated system of claim 14, wherein, the nucleic acid shuffling or mutagenesis module performs one or more of: StEP PCR, uracil incorporation or chain termination.

23. The device or integrated system of claim 14, or 20, wherein the nucleic acid shuffling module comprises an identification portion, which identification portion identifies one or more nucleic acid portion or subportion.

24. The device or integrated system of claim 14 or 20, wherein the nucleic acid shuffling module comprises a fragment length purification portion, which fragment length purification portion purifies selected length fragments of the nucleic acid fragments.

25. The device or integrated system of claim 20, wherein the nucleic acid shuffling module permits hybridization of the nucleic acid fragments and wherein the nucleic acid shuffling module comprises a polymerase which elongates the hybridized nucleic acid.

26. The device or integrated system of claim 25, wherein the nucleic acid shuffling module combines one or more translation or transcription control sequence into the resulting elongated nucleic acid.

27. The device or integrated system of claim 26, wherein the one or more translation or transcription control sequence is combined into the resulting elongated nucleic acid using the polymerase, or a ligase, or both the polymerase and the ligase.

28. The device or integrated system of claim 25, wherein the nucleic acid shuffling module separates, identifies, purifies or immobilizes the resulting elongated nucleic acid.

29. The device or integrated system of claim 25, wherein the nucleic acid shuffling module determines a recombination frequency or a length, or both a recombination frequency and a length, for the resulting elongated nucleic acids.

30. The device or integrated system of claim 25, wherein the nucleic acid shuffling module determines nucleic acid length by detecting incorporation of one or more labeled nucleic acid or nucleotide into the resulting elongated nucleic acid.

Sub  
AI

09760010-011001

31. The device or integrated system of claim 25, wherein the nucleic acid shuffling module determines nucleic acid length by detecting one or more label associated with the resulting elongated nucleic acid.

32. The device or integrated system of claim 30, wherein the label is a dye, radioactive label, biotin, digoxin, or a fluorophore.

33. The device or integrated system of claim 25, wherein the nucleic acid shuffling module determines nucleic acid length with a fluorogenic 5' nuclease assay.

34. The device or integrated system of claim 1, wherein the physical or logical array of reaction mixtures is incorporated into a microscale device, or wherein at least one of the reaction mixtures is incorporated into a microscale device, or wherein the one or more shuffled or mutagenized nucleic acids or the one or more transcribed shuffled or mutagenized nucleic acids is found within a microscale device, or wherein the one or more in vitro translation reagents is found within a microscale device.

35. The device or integrated system of claim 25, wherein the nucleic acid shuffling module comprises one or more microscale channel through which a shuffling reagent or product is flowed.

36. The device or integrated system of claim 35, wherein the channel is integrated in a chip.

37. The device or integrated system of claim 35, wherein liquid flow through the device is mediated by capillary flow, differential pressure between one or more inlets and outlets, electroosmosis, hydraulic or mechanical pressure, or peristalsis.

38. The device or integrated system of claim 25, wherein the nucleic acid fragments are contacted in a single pool.

39. The device or integrated system of claim 25, wherein the nucleic acid fragments are contacted in multiple pools.

Sub  
A1

09760010.014001

40. The device or integrated system of claim 25, wherein the nucleic acid shuffling module dispenses the resulting elongated nucleic acids into one or more multiwell plates, or onto one or more solid substrates, or into one or more microscale systems, or into one or more containers.

41. The device or integrated system of claim 25, wherein the nucleic acid shuffling module pre-dilutes the resulting elongated nucleic acids and dispenses them into one or more multiwell plates.

42. The device or integrated system of claim 25, wherein the nucleic acid shuffling module dispenses the resulting elongated nucleic acids into one or more multiwell plates at a selected density per well of the elongated nucleic acids.

43. The device or integrated system of claim 25, wherein the nucleic acid shuffling module dispenses the resulting elongated nucleic acids into one or more master multiwell plates and PCR amplifies the resulting master array of elongated nucleic acids to produce an amplified array of elongated nucleic acids, the shuffling module further comprising a array copy system which transfers aliquots from the wells of the one or more master multiwell plates to one or more copy multiwell plates.

44. The device or integrated system of claim 43, wherein an extent of PCR amplification is determined by one or more technique selected from: an incorporation of a label into one or more amplified elongated nucleic acid, and a fluorogenic 5' nuclease assay.

45. The device or integrated system of claim 43, wherein the array of reaction mixtures is formed by separate or simultaneous addition of an in vitro transcription reagent and an in vitro translation reagent to the one or more copy multiwell plates, or to a duplicate set thereof, wherein the elongated nucleic acids comprise the one or more shuffled nucleic acids.

46. The device or integrated system of claim 1, further comprising one or more sources of one or more nucleic acids, the one or more sources collectively or individually comprising a first population of nucleic acids, wherein the shuffled nucleic

Sub  
A1

09760010-011001

acids are produced by recombining the one or more members of the first population of nucleic acids.

Sub  
A1

47. The device or integrated system of claim 46, the one or more sources of nucleic acids comprising at least one nucleic acid selected from: a synthetic nucleic acid, a DNA, an RNA, a DNA analogue, an RNA analogue, a genomic DNA, a cDNA, an mRNA, a DNA generated by reverse transcription, an nRNA, an aptamer, a polysome associated nucleic acid, a cloned nucleic acid, a cloned DNA, a cloned RNA, a plasmid DNA, a phagemid DNA, a viral DNA, a viral RNA, a YAC DNA, a cosmid DNA, a fosmid DNA, a BAC DNA, a P1-mid, a phage DNA, a single-stranded DNA, a double-stranded DNA, a branched DNA, a catalytic nucleic acid, an antisense nucleic acid, an in vitro amplified nucleic acid, a PCR amplified nucleic acid, an LCR amplified nucleic acid, a Q $\beta$ -replicase amplified nucleic acid, an oligonucleotide, a nucleic acid fragment, a restriction fragment and a combination thereof.

48. The device or integrated system of claim 46, further comprising a population destination region, wherein, during operation of the device, one or more members of the first population are moved from the one or more sources of the one or more nucleic acids to the one or more destination regions.

49. The device or integrated system of claim 48, further comprising nucleic acid movement means for moving the one or more members from the one or more sources of the one or more nucleic acids to the one or more destination regions.

50. The device or integrated system of claim 46, 48, or 49 further comprising a source of an in vitro transcription reagent or an in vitro translation reagent, wherein, during operation of the device, the in vitro transcription reagent or an in vitro translation reagent is flowed into contact with the members of the first population.

51. The device of claim 50, wherein the members of the first population are fixed at the one or more sources of one or more nucleic acids or at the one or more destination regions.

09750010-011001

Sub  
A1

52. The device or integrated system of claim 49, wherein the nucleic acid movement means comprises one or more movement means selected from: a fluid pressure modulator, an electrokinetic fluid force modulator, a thermokinetic modulator, a capillary flow mechanism, a centrifugal force modulator, a robotic armature, a pipettor, a conveyor mechanism, a peristaltic pump or mechanism, a magnetic field generator, an electric field generator, and one or more fluid flow path.

53. The device or integrated system of claim 48, the one or more sources of nucleic acids, or the one or more population destination regions comprising one or more member selected from: a solid phase array, a liquid phase array, a container, a microtiter tray, a microtiter tray well, a microfluidic component, a microfluidic chip, a test tube, a centrifugal rotor, a microscope slide, an organism, a cell, a tissue, a liposome, a detergent particle, and a combination thereof.

54. The device or integrated system of claim 45, wherein, during operation of the device, the first population of nucleic acids is arranged into one or more physical or logical recombinant nucleic acid arrays.

55. The device or integrated system of claim 54, further comprising a duplicate of at least one of the one or more physical or logical recombinant nucleic acid arrays.

56. The device or integrated system of claim 45 or 54, further comprising one or more recombination modules which move one or more members of the first population of nucleic acids into contact with one another, thereby facilitating recombination of the first population of nucleic acids.

57. The device or integrated system of claim 1, further comprising one or more reaction mixture arraying modules, which arraying modules move one or more of the one or more shuffled nucleic acids or the one or more transcribed shuffled nucleic acids or the in vitro translation reactant components into one or more spatial positions, thereby placing the one or more shuffled nucleic acids or the one or more transcribed



shuffled nucleic acids or the in vitro translation reactant component into locations in the array of reaction mixtures.

58. The device of integrated system of claim 1, further comprising a shuffled nucleic acid master array, which master array physically or logically corresponds to positions of the shuffled nucleic acids in the reaction mixture array.

59. The device or integrated system of claim 58, further comprising a nucleic acid amplification module, which module amplifies members of the shuffled nucleic acid master array, or a duplicate thereof.

60. The device or integrated system of claim 59, the amplification module comprising a heating or cooling element.

61. The device or integrated system of claim 59, the amplification module comprising a DNA micro-amplifier.

62. The device or integrated system of claim 59, the amplification module comprising a DNA micro-amplifier, the micro-amplifier comprising one or more of: a programmable resistor, a micromachined zone heating chemical amplifier, a Peltier solid state heat pump, a heat pump, a heat exchanger, a hot air blower, a resistive heater, a refrigeration unit, a heat sink, or a Joule Thompson cooling device.

63. The device or integrated system of claim 59, further comprising a duplicate amplified array, which duplicate amplified array comprises amplicons of the nucleic acid master array, or a duplicate thereof.

64. The device or integrated system of claim 58, wherein, during operation of the device, the array of reaction mixtures produces an array of reaction mixture products, the device or integrated system further comprising one or more product identification or purification modules, which product identification modules identify one or more members of the array of reaction products.

65. The device or integrated system of claim 64, wherein the product identification or purification modules comprise one or more of: a gel, a polymeric

Sub  
A1

09760010-011001

Sub  
A1

09750010-011001

solution, a liposome, a microemulsion, a microdroplet, an affinity matrix, a plasmon resonance detector, a BIACORE, a GC detector, an ultraviolet or visible light sensor, an epifluorescence detector, a fluorescence detector, a fluorescent array, a CCD, a digital imager, a scanner, a confocal imaging device, an optical sensor, a FACS detector, a micro-FACS unit, a temperature sensor, a mass spectrometer, a stereo-specific product detector, an Elisa reagent, an enzyme, an enzyme substrate, an antibody, an antigen, a refractive index detector, a polarimeter, a pH detector, a pH-stat device, an ion selective sensor, a calorimeter, a film, a radiation sensor, a Geiger counter, a scintillation counter, a particle counter, an H<sub>2</sub>O<sub>2</sub> detection system, an electrochemical sensor, ion/gas selective electrodes, and capillary electrophoresis.

66. The device or integrated system of claim 64, wherein the one or more reaction product array members are moved into proximity to the product identification module, or wherein the product identification module performs an xyz translation, thereby moving the product identification module proximal to the array of reaction products.

67. The device or integrated system of claim 66, wherein the one or more reaction product array members are flowed into proximity to the product identification module, wherein an in-line purification system purifies the one or more reaction product array members from associated materials.

68. The device or integrated system of claim 64, wherein the reaction products comprise one or more polypeptide, one or more nucleic acid, one or more catalytic RNA, or one or more biologically active RNA.

69. The device or integrated system of claim 68, wherein the one or more catalytic RNA is a ribozyme, or wherein the biologically active RNA is an anti-sense RNA.

70. The device or integrated system of claim 68, wherein the device further comprises a source of one or more lipid, which one or more lipid is flowed into contact with the one or more polypeptide, or wherein the lipid is flowed into contact with

Sub  
AI

the physical or logical array of reaction mixtures, or wherein the lipid is flowed into contact with the one or more transcribed shuffled or mutagenized nucleic acids, thereby producing one or more liposomes or micelles comprising the polypeptide, reaction mixture components, or one or more transcribed shuffled or mutagenized nucleic acids.

71. The device or integrated system of claim 64, wherein the reaction products comprise one or more polypeptide and wherein the device further comprises one or more protein refolding reagent, which refolding reagent is flowed into contact with the one or more polypeptide.

72. The device or integrated system of claim 71, wherein the refolding reagent comprises one or more of: guanidine, guanidinium, urea, a detergent, a chelating agent, DTT, DTE, or a chaperonin.

73. The device or integrated system of claim 64, the product identification or purification modules comprising one or more of: a protein detector, or protein purification means.

74. The device or integrated system of claim 64, the product identification or purification modules comprising an instruction set for discriminating between members of the array of reaction products based upon one or more of: a physical characteristic of the members, an activity of the members, or concentrations of the members.

75. The device or integrated system of claim 64, further comprising a secondary product array produced by re-arraying members of the reaction product array such that the secondary product array has a selected concentration of product members in the secondary product array.

76. The device or integrated system of claim 75, wherein the selected concentration is approximately the same for a plurality of product members in the secondary product array.

Sub  
A1

00760010 011001

77. The device or integrated system of claim 64, further comprising an instruction set for determining a correction factor which accounts for variation in polypeptide concentration at different positions in the amplified physical or logical array of polypeptides.

78. The device or integrated system of claim 64 or 75, further comprising a substrate addition module which substrate addition module adds one or more substrate to a plurality of members of the product array or the secondary product array.

79. The device of claim 78, further comprising a substrate conversion detector which monitors formation of a product produced by contact between the one or more substrate and one or more of the plurality of members of the product array or the secondary product array.

80. The device of claim 79, wherein formation of the product or disappearance of substrate is monitored indirectly.

81. The device of claim 79, wherein formation of the product or disappearance of substrate is monitored by monitoring loss of the substrate over time.

82. The device of claim 79, wherein formation of the product or disappearance of substrate is monitored enantioselectively, regioselectively or stereoselectively.

83. The device of claim 82, wherein formation of the product or disappearance of substrate is monitored by adding at least one isomer, enantiomer or stereoisomer in substantially pure form, which substantially pure form is independent of other potential isomers.

84. The device of claim 79, wherein formation of the product is monitored by detecting formation of peroxide, protons, or halides, or reduced or oxidized cofactors.

Sub  
A1

85. The device of claim 79, wherein formation of the product is monitored by detecting changes in heat or entropy which result from contact between the substrate and the product, or by detecting changes in mass, charge, fluorescence, epifluorescence, by chromatography, luminescence or absorbance, of the substrate or the product, which result from contact between the substrate and the product.

86. The device or integrated system of claim 64, the device or integrated system further comprising an array correspondence module, which array correspondence module identifies, determines or records the location of an identified product in the array of reaction mixture products which is identified by the one or more product identification modules, or which array correspondence module determines or records the location of at least a first nucleic acid member of the shuffled nucleic acid master array, or a duplicate thereof, or of an amplified duplicate array, which member corresponds to the location of one or more member of the array of reaction products.

87. The device or integrated system of claim 73, further comprising one or more secondary selection module, which secondary selection module selects at least the first member for further recombination, which selection is based upon the location of a product identified by the product identification modules.

88. The device or integrated system of claim 64, further comprising a screening or selection module, the module comprising one or more of:

an array reader, which reader detects one or more member of the array of reaction products;

an enzyme which converts one or more member of the array of reaction products into one or more detectable products;

a substrate which is converted by the one or more member of the array of reaction products into one or more detectable products;

a cell which produces a detectable signal upon incubation with the one or more member of the array of reaction products;

a reporter gene which is induced by one or more member of the array of reaction products;

Sub  
A1

a promoter which is induced by one or more member of the array of reaction products, which promoter directs expression of one or more detectable products;  
or

an enzyme or receptor cascade which is induced by the one or more member of the array of reaction products.

89. The device or integrated system of claim 87, further comprising a secondary recombination module, which module physically contacts the first member, or an amplicon thereof, to an additional member of the shuffled nucleic acid master array, or the duplicate thereof, or the amplified duplicate array, thereby permitting physical recombination between the first and additional members.

90. The device or integrated system of claim 1, further comprising a DNA fragmentation module and a recombination region, which DNA fragmentation module comprises one or more of: a nuclease, a mechanical shearing device, a polymerase, a random primer, a directed primer, a nucleic acid cleavage reagent, a chemical nucleic acid chain terminator, or an oligonucleotide synthesizer, wherein, during operation of the device, fragmented DNAs produced in the DNA fragmentation module are recombined in the recombination region to produce the one or more shuffled nucleic acids.

91. The device or integrated system of claim 1, further comprising a module which performs one or more of: error prone PCR, site saturation mutagenesis, or site-directed mutagenesis.

92. The device or integrated system of claim 1, further comprising a data structure embodied in a computer, an analog computer, a digital computer, or a computer readable medium, which data structure corresponds to the one or more shuffled nucleic acids.

93. The device or integrated system of claim 1, wherein the one or more reaction mixtures comprise one or more shuffled nucleic acids arranged in a microtiter tray at an average of approximately 0.1-100 shuffled nucleic acids per well.

Sub  
A1

94. The device or integrated system of claim 1, wherein the one or more reaction mixtures comprise one or more shuffled nucleic acids arranged in a microtiter tray at an average of approximately 1-5 shuffled nucleic acids per well.

95. The device or integrated system of claim 1, further comprising a diluter, which diluter pre-dilutes the concentration of the one or more shuffled or mutated nucleic acids prior to addition of the shuffled or mutant nucleic acids to the reaction mixtures.

96. The device or integrated system of claim 95, wherein the concentration of the one or more shuffled nucleic acids is about 0.01 to 100 molecules per microliter.

97. The device or integrated system of claim 1, wherein the reaction mixtures are produced by adding the in vitro translation reactant and, optionally, an in vitro transcription reagents, to a duplicate shuffled or mutated nucleic acid array, which duplicate shuffled or mutated nucleic acid array is duplicated from a master array of the shuffled or mutated nucleic acids produced by spatially or logically separating members of a population of the shuffled or mutated nucleic acids to produce a physical or logical array of the shuffled or mutated nucleic acids, by one or more arraying technique selected from:

(i) lyophilizing members of the population of shuffled nucleic acids on a solid surface, thereby forming a solid phase array;

(ii) chemically coupling members of the population of shuffled nucleic acids to a solid surface, thereby forming a solid phase array;

(iii) rehydrating members of the population of shuffled nucleic acids on a solid surface, thereby forming a liquid phase array;

(iv) cleaving chemically coupled members of the population of shuffled nucleic acids from a solid surface, thereby forming a liquid phase array;

(v) accessing one or more physically separated logical array members from one or more sources of shuffled nucleic acids and flowing the physically separated logical

array members to one or more destination, the one or more destinations constituting a logical array of the shuffled nucleic acids; and,

(vi) printing members of a population of shuffled nucleic acids onto a solid material to form a solid phase array.

**98.** The device or integrated system of claim 1, wherein the one or more shuffled nucleic acids are produced by synthesizing a set of overlapping oligonucleotides, or by cleaving a plurality of homologous nucleic acids to produce a set of cleaved homologous nucleic acids, or both, and permitting recombination to occur between the set of overlapping oligonucleotides, the set of cleaved homologous nucleic acids, or both the set of overlapping oligonucleotides and the set of cleaved homologous nucleic acids.

**99.** The device or integrated system of claim 1, wherein greater than about 1% of the physical or logical array of reaction mixtures comprise shuffled or mutant nucleic acids having one or more base changes relative to a parental nucleic acid.

**100.** A diversity generation device, comprising

- (i) a programmed thermocycler; and,
- (ii) a fragmentation module operably coupled to the programmed thermocycler.

**101.** The diversity generation device of claim **100**, wherein the programmed thermocycler comprises a thermocycler operably coupled to a computer, which computer comprises one or more instruction set, which one or more instruction set does one or more of:

calculates an amount of uracil and an amount of thymidine for use in the programmed thermocycler;

calculates one or more crossover region between two or more parental nucleotides;

calculates an annealing temperature;

calculates an extension temperature; or

selects one or more parental nucleic acid sequence.

Sub  
A1

09750030 011001



**102.** The diversity generation device of claim **101**, wherein the one or more instruction set receives user input data and sets up one or more cycle to be performed by the programmed thermocycler.

**103.** The diversity generation device of claim **102**, wherein the input data comprises one or more of: one or more parental nucleic acid sequence, a desired crossover frequency, an extension temperature, or an annealing temperature.

**104.** The diversity generation device of claim **101**, wherein the one or more instruction set calculates the amount of uracil and the amount of thymidine based on a desired fragment size.

**105.** The diversity generation device of claim **103**, wherein the one or more instruction set directs the one or more cycle on the diversity generation device, which one or more cycle:

- (a) amplifies the one or more parental nucleic acid sequence;
- (b) fragments the one or more parental nucleic acid sequence to produce one or more nucleic acid fragment;
- (c) reassembles the one or more nucleic acid fragment to produce one or more shuffled nucleic acid; and,
- (d) amplifies the one or more shuffled nucleic acid.

**106.** The diversity generation device of claim **105**, wherein step (a) comprises amplifying the one or more parental nucleic acid sequence in the presence of uracil.

**107.** The diversity generation device of claim **105**, wherein the one or more cycle pauses between step (a) and step (b) to allow addition of one or more fragmentation reagent.

**108.** The diversity generation device of claim **101**, wherein the one or more instruction set performs one or more calculation based on one or more theoretical prediction of a nucleic acid melting temperature or on one or more set of empirical data,

which empirical data comprises a comparison of one or more nucleic acid melting temperature.

**109.** The diversity generation device of claim **105**, wherein the one or more instruction set instructs the fragmentation module to fragment the parental nucleic acids to produce one or more nucleic acid fragments having a desired mean fragment size.

**110.** The diversity generation device of claim **100**, wherein the programmed thermocycler comprises a thermocycler and software for performing one or more shuffling calculations, which software is embodied on a web page or is installed directly in the thermocycler.

**111.** The diversity generation device of claim **100**, wherein the fragmentation module fragments one or more parental nucleic acids by sonication, DNase II digestion, random primer extension, or uracil incorporation and treatment with one or more uracil cleavage enzyme.

**112.** A diversity generation device comprising:

- (i) a computer, which computer comprises at least a first instruction set for creating one or more nucleic acid fragment sequence from one or more parental nucleic acid sequence;
- (ii) a synthesizer module, which synthesizer module synthesizes the one or more nucleic acid fragment sequence; and,
- (iii) a thermocycler, which thermocycler generates one or more diverse sequence from the one or more nucleic acid fragment sequence.

**113.** The diversity generation device of claim **112**, wherein the first instruction set limits or expands diversity of the one or more nucleic acid fragment sequence by adding or removing one or more amino acid having similar diversity; selecting a frequently used amino acid at one or more specific position; using one or more sequence activity calculation; using a calculated overlap with one or more

additional oligonucleotide; based on an amount of degeneracy, or based on a melting temperature.

**114.** The diversity generation device of claim **112**, wherein the synthesizer module comprises a microarray oligonucleotide synthesizer.

**115.** The diversity generation device of claim **114**, wherein the synthesizer module comprises an ink-jet printer head based oligonucleotide synthesizer.

**116.** The diversity generation device of claim **112**, wherein the synthesizer module synthesizes the one or more nucleic acid fragment sequences on a solid support.

**117.** The diversity generation device of claim **112**, wherein the synthesizer module uses one or more mononucleotide coupling reactions or one or more trinucleotide coupling reactions to synthesize the one or more nucleic acid fragment sequence.

**118.** The diversity generation device of claim **112**, wherein the thermocycler performs an assembly/rescue PCR reaction.

**119.** The diversity generation device of claim **118**, wherein the computer comprises at least a second instruction set, which second instruction set determines at least a first set of conditions for the assembly/rescue PCR reaction.

**120.** The diversity generation device of claim **112**, the device further comprising a screening module for screening the one or more diverse sequence for a desired characteristic.

**121.** The diversity generation device of claim **120**, wherein the screening module comprises a high-throughput screening module.

**122.** A diversity generation kit comprising:

- (i) the diversity generation device of claim **100** or claim **112**; and,
- (ii) one or more reagent for diversity generation.

**123.** The diversity generation kit of claim **122**, wherein the reagents comprise E coli., a PCR reaction mixture comprising a mixture of uracil and thymidine, one or more uracil cleaving enzyme, and a PCR reaction mixture comprising standard dNTPs.

**124.** The diversity generation kit of claim **123**, wherein the one or more uracil cleaving enzyme comprises a uracil glycosidase and an endonuclease.

**125.** The diversity generation kit of claim **123**, wherein the mixture of uracil and thymidine comprises a desired ratio of uracil to thymidine, which desired ratio is calculated by the diversity generation device.

**126.** The diversity generation kit of claim **122**, wherein the one or more reagents for diversity generation comprise at least a first artificially evolved enzyme. The diversity generation kit of claim **126**, wherein the at least first artificially evolved enzyme comprises an artificially evolved polymerase.

**127.** The diversity generation kit of claim **122**, further comprising one or more of: packaging materials, a container adapted to receive the device or reagent, or instructional materials for use of the device.

**128.** A method of processing shuffled or mutagenized nucleic acids, the method comprising:

(a) providing a physical or logical array of reaction mixtures, a plurality of the reaction mixtures comprising one or more member of a first population of nucleic acids, the first population of nucleic acids comprising one or more shuffled nucleic acids, or one or more transcribed shuffled nucleic acids, or one or more mutagenized nucleic acid or one or more transcribed mutagenized nucleic acids wherein a plurality of the plurality of reaction mixtures further comprise an in vitro translation reactant; and,

(b) detecting one or more in vitro translation products produced by a plurality of members of the physical or logical array of reaction mixtures.

**129.** The physical or logical array or reaction mixtures produced by the method of claim **128**.

**130.** The method of claim 128, wherein the array of reaction mixtures comprises a solid phase or a liquid phase array of one or more of: the one or more shuffled or mutagenized nucleic acids, the one or more transcribed shuffled nucleic acids, or the one or more in vitro translation reagents.

**131.** The method of claim 128, wherein the one or more shuffled nucleic acids or the one or more mutagenized nucleic acids are homologous.

**132.** The method of claim 128, wherein the one or more transcribed shuffled nucleic acid or the one or more transcribed mutagenized nucleic acid is an mRNA, a catalytic RNA or a biologically active RNA.

**133.** The method of claim 128, wherein the one or more in vitro translation reagents comprise one or more of: a reticulocyte lysate, a rabbit reticulocyte lysate, a wheat germ in vitro translation mixture, or an *E coli* lysate.

**134.** The method of claim 128, further comprising providing one or more in vitro transcription reagents to the plurality of members of the physical or logical array of reaction mixtures.

**135.** The method of claim 134, wherein the in vitro transcription reagents comprises one or more of: a HeLa nuclear extract in vitro transcription component, an SP6 polymerase, a T3 polymerase or a T7 RNA polymerase.

**136.** The method of claim 128, wherein the one or more shuffled nucleic acids are produced in an automatic DNA shuffling module, the method comprising inputting DNAs or character strings corresponding to input DNAs into the DNA shuffling module and accepting output DNAs from the DNA shuffling module, which output DNAs comprise the one or more shuffled nucleic acids in the reaction mixture array.

**137.** The method of claim 136, comprising fragmenting the input DNA in the DNA shuffling module to produce DNA fragments, or providing the input DNAs to comprise cleaved or synthetic DNA fragments.

**138.** The method of claim 136, or 137, comprising purifying DNA fragments of a selected length in the DNA shuffling module.

**139.** The method of claim 138, comprising hybridizing the resulting purified DNA fragments and elongating the resulting hybridized DNA fragments with a polymerase.

**140.** The method of claim 139, further comprising separating, identifying, cloning or purifying the resulting elongated DNAs.

**141.** The method of claim 139, further comprising determining a recombination frequency or a length, or both a recombination frequency and a length for the resulting elongated DNAs.

**142.** The method of claim 139, further comprising determining a length of the resulting elongated DNAs by detecting incorporation of one or more labeled nucleic acid or nucleotide into the elongated DNAs.

**143.** The method of claim 142, wherein the label is a dye, radioactive label, or a fluorophore.

**144.** The method of claim 139, comprising determining the length of the resulting elongated DNAs with a fluorogenic 5' nuclease assay.

**145.** The method of claim 139, comprising flowing a shuffling reagent or product through a microscale channel in the DNA shuffling module.

**146.** The method of claim 139, wherein the DNA fragments are contacted in a single pool.

**147.** The method of claim 139, wherein the DNA fragments are contacted in multiple pools.

**148.** The method of claim 139, further comprising dispensing the resulting elongated DNAs into one or more multiwell plates.

**149.** The method of claim 139, further comprising dispensing the resulting elongated DNAs into one or more multiwell plates at a selected density per well of the elongated DNAs.

**150.** The method of claim 139, further comprising dispensing the resulting elongated DNAs into one or more master multiwell plates and PCR amplifying the resulting master array of elongated nucleic acids to produce an amplified array of elongated nucleic acids, the shuffling module comprising a array copy system which transfers aliquots from the wells of the one or more master multiwell plates to one or more copy multiwell plates.

**151.** The method of claim 150, comprising determining an extent of PCR amplification by one or more technique selected from: incorporation of a label into one or more amplified elongated nucleic acid, and applying a fluorogenic 5' nuclease assay.

**152.** The method of claim 150, wherein the array of reaction mixtures is formed by separate or simultaneous addition of an in vitro transcription reagents and an in vitro translation reactant to the one or more copy multiwell plates, or to a duplicate set thereof, wherein the elongated DNAs comprise the one or more shuffled nucleic acids.

**153.** The method of claim 128, wherein the array of reaction mixtures produces an array of reaction mixture products.

**154.** The method of claim 153, wherein the reaction products comprise one or more polypeptide.

**155.** The method of claim 153, wherein the reaction products comprise one or more polypeptide, the method further comprising re-folding the one or more polypeptide by contacting the one or more polypeptide with a refolding reagent.

**156.** The method of claim 155, wherein the refolding reagent comprises one or more of: guanidine, urea, DTT, DTE, or a chaperonin.

**157.** The method of claim 153, comprising moving the one or more reaction product array members into proximity to a product identification module, or

moving a product identification module into proximity to the reaction product array members.

**158.** The method of claim 153, wherein the one or more reaction product array members are flowed into proximity to a product identification module, the method further comprising in-line purification of the one or more reaction product array members.

**159.** The method of claim 153, further comprising contacting the one or more polypeptide with one or more lipid to produce one or more liposome or micelle, which liposome or micelle comprises the one or more polypeptide.

**160.** The method of claim 153, further comprising one or more of:  
reading the array of reaction mixture products with an array reader, which reader detects one or more member of the array of reaction products;  
converting one or more member of the array of reaction products with an enzyme into one or more detectable products;  
converting one or more substrates by the one or more member of the array of reaction products into one or more detectable products;  
contacting a cell to one or more member of the array of reaction products, which cell or reaction product, or both, produce a detectable signal upon contacting the one or more member of the array of reaction products;  
inducing a reporter gene with one or more member of the array of reaction products;  
inducing a promoter with one or more member of the array of reaction products, which promoter directs expression of one or more detectable products; or  
inducing an enzyme or receptor cascade with one or more member of the array of reaction products, which cascade is induced by the one or more member of the array of reaction products.

**161.** A method of recombining members of a physical or logical array of nucleic acids, the method comprising:

(a) providing at least a first population of nucleic acids, or



(b) providing a data structure comprising character strings corresponding to the first population of nucleic acids;

(c) recombining one or more members of the first population of nucleic acids, thereby providing a first population of recombinant nucleic acids, or

(d) recombining one or more of the character strings corresponding to one or more members of the first population of nucleic acids, thereby providing a population of character strings corresponding to the first population of recombinant nucleic acids, and converting the population of character strings corresponding to the first population of recombinant nucleic acids into the first population of recombinant nucleic acids, thereby providing the first population of recombinant nucleic acids;

(e) spatially or logically separating members of the population of recombinant nucleic acids to produce a physical or logical array of recombinant nucleic acids and amplifying the recombinant nucleic acids in the physical or logical array of recombinant nucleic acids in vitro to provide an amplified physical or logical array of recombinant nucleic acids, or,

(f) in vitro amplifying members of the population of recombinant nucleic acids and physically or logically separating the population of recombinant nucleic acids to produce an amplified physical or logical array of recombinant nucleic acids.

**162.** The method of claim 161, further comprising:

(g) screening the amplified physical or logical array of recombinant nucleic acids, or a duplicate thereof, for a desired property.

**163.** The method of claim 161, wherein the data structure is embodied in a computer, an analog computer, a digital computer, or a computer readable medium.

**164.** The method of claim 161, wherein spatially or logically separating members of the population of recombinant nucleic acids to produce a physical or logical array of recombinant nucleic acids or amplified recombinant nucleic acids comprises plating the nucleic acids in a microtiter tray at an average of approximately 0.1-10 array members per well.

09750070-011001

**165.** The method of claim 161, wherein spatially or logically separating members of the population of recombinant nucleic acids to produce a physical or logical array of recombinant nucleic acids comprises plating the nucleic acids in a microtiter tray at an average of approximately 1-5 array members per well.

**166.** The method of claim 161, wherein spatially or logically separating the members of the population of recombinant nucleic acids comprises diluting the members of the population with a buffer.

**167.** The method of claim 161, wherein the concentration of the population of recombinant nucleic acids is about 0.01 to 100 molecules per microliter.

**168.** The method of claim 161, wherein spatially or logically separating members of the population of recombinant nucleic acids to produce a physical or logical array of recombinant nucleic acids comprises one or more of:

- (i) lyophilizing members of the population of recombinant nucleic acids on a solid surface, thereby forming a solid phase array;
- (ii) chemically coupling members of the population of recombinant nucleic acids to a solid surface, thereby forming a solid phase array;
- (iii) rehydrating members of the population of recombinant nucleic acids on a solid surface, thereby forming a liquid phase array;
- (iv) cleaving chemically coupled members of the population of recombinant nucleic acids from a solid surface, thereby forming a liquid phase array; or,
- (v) accessing one or more physically separated logical array members from one or more sources of recombinant nucleic acids and flowing the physically separated logical array members to one or more destination.

**169.** A method of recombining members of a physical or logical array of nucleic acids, the method comprising:

- (a) providing at least a first population of nucleic acids arranged in a physical or logical array;

(b) recombining one or more members of the first population of nucleic acids with one or more additional nucleic acid, thereby providing a first physical or logical array comprising a population of recombinant nucleic acids;

(c) amplifying the recombinant nucleic acids in the physical or logical array of recombinant nucleic acids in vitro to provide an amplified physical or logical array of recombinant nucleic acids; and,

(g) screening the first or amplified physical or logical array of recombinant nucleic acids, or a duplicate thereof, for a desired property.

**170.** The method of claim 128 or 169, wherein the first population of nucleic acids or the population of recombinant nucleic acids are arranged in a physical or logical matrix at an average of approximately 0.1-10 array members per array position.

**171.** The method of claim 128 or 169, wherein the first population of nucleic acids or the population of recombinant nucleic acids are arranged in a physical or logical matrix at an average of approximately 0.5-5 array members per array position.

**172.** The method of claim 128 or 169, wherein the first population of nucleic acids or the population of recombinant nucleic acids comprise a solid phase or a liquid phase array.

**173.** The method of claim 128 or 169, wherein the first population of nucleic acids is provided by one or more of:

synthesizing a set of overlapping oligonucleotides, cleaving a plurality of homologous nucleic acids to produce a set of cleaved homologous nucleic acids, step PCR of one or more target nucleic acid, uracil incorporation and cleavage during copying of one or more target nucleic acids, and incorporation of a cleavable nucleic acid analogue into a target nucleic acid and cleavage of the resulting target nucleic acid; or,

wherein the set of overlapping oligonucleotides or the set of cleaved homologous nucleic acids are flowed into one or more selected physical locations.

**174.** The method of claim 128, 161 or 169, wherein the first population of nucleic acids is provided by synthesizing a set of overlapping oligonucleotides, by

cleaving a plurality of homologous nucleic acids to produce a set of cleaved homologous nucleic acids, or both.

**175.** The method of claim 128, 161 or 169, wherein the first population of nucleic acids is provided by sonicating, cleaving, partially synthesizing, random primer extending or directed primer extending one or more of: a synthetic nucleic acid, a DNA, an RNA, a DNA analogue, an RNA analogue, a genomic DNA, a cDNA, an mRNA, a DNA generated by reverse transcription, an nRNA, an aptamer, a polysome associated nucleic acid, a cloned nucleic acid, a cloned DNA, a cloned RNA, a plasmid DNA, a phagemid DNA, a viral DNA, a viral RNA, a YAC DNA, a cosmid DNA, a fosmid DNA, a BAC DNA, a P1-mid, a phage DNA, a single-stranded DNA, a double-stranded DNA, a branched DNA, a catalytic nucleic acid, an antisense nucleic acid, an in vitro amplified nucleic acid, a PCR amplified nucleic acid, an LCR amplified nucleic acid, a Q $\beta$ -replicase amplified nucleic acid, an oligonucleotide, a nucleic acid fragment, a restriction fragment or a combination thereof.

**176.** The method of claim 175, wherein the first population of nucleic acids is further provided by purifying one or more member of the first population of nucleic acids.

**177.** The method of claim 128, 161 or 169, wherein the first population of nucleic acids is provided by transporting one or more members of the population from one or more sources of one or more members of the first population to one or more destinations of the one or more members of the first population of nucleic acids.

**178.** The method of claim 177, wherein said transporting comprises flowing the one or more members from the source to the destination.

**179.** The method of claim 177, the one or more sources of nucleic acids comprising one or more of: a solid phase array, a liquid phase array, a container, a microtiter tray, a microtiter tray well, a microfluidic chip, a test tube, a centrifugal rotor, a microscope slide, or a combination thereof.

180. The method of claim 150, 161 or 169, wherein amplifying the recombinant nucleic acids in the physical or logical array of recombinant nucleic acids, or amplifying the elongated nucleic acids in the master array comprises one or more amplification technique selected from: PCR, LCR, SDA, NASBA, TMA and Q $\beta$ -replicase amplification.

181. The method of claim 150, 161 or 169, wherein amplifying the recombinant nucleic acids in the physical or logical array or amplifying the elongated nucleic acids in the master array comprises heating or cooling the physical or logical array or the master array, or a portion thereof.

182. The method of claim 150, 161 or 169, wherein amplifying the recombinant nucleic acids in the physical or logical array or amplifying the elongated nucleic acids in the master array comprises incorporating one or more transcription or translation control subsequence into one or more of:

the elongated nucleic acids, the recombinant nucleic acids in the physical or logical array, an intermediate nucleic acid produced using the elongated nucleic acids or the recombinant nucleic acids in the physical or logical array as a template, or a partial or complete copy of the elongated nucleic acids or the recombinant nucleic acids in the physical or logical array.

183. The method of claim 182, wherein the one or more transcription or translation control subsequence is ligated to into one or more of:

the elongated nucleic acids, the recombinant nucleic acids in the physical or logical array, an intermediate nucleic acid produced using the elongated nucleic acids or the recombinant nucleic acids in the physical or logical array as a template, or a partial or complete copy of the elongated nucleic acids or the recombinant nucleic acids in the physical or logical array.

184. The method of claim 182, wherein the one or more transcription or translation control subsequence is hybridized or partially hybridized to one or more of:

the elongated nucleic acids, the recombinant nucleic acids in the physical or logical array, an intermediate nucleic acid produced using the elongated nucleic acids or

the recombinant nucleic acids in the physical or logical array as a template, or a partial or complete copy of the elongated nucleic acids or the recombinant nucleic acids in the physical or logical array.

**185.** The method of claim 181, wherein the recombinant nucleic acids in the physical or logical array or the elongated nucleic acids in the master array are amplified in a DNA micro-amplifier.

**186.** The method of claim 185, wherein the micro-amplifier comprises one or more of: a programmable resistor, a micromachined zone heating chemical amplifier, a chemical denaturation device, an electrostatic denaturation device, or a microfluidic electrical fluid resistance heating device.

**187.** The method of claim 181, wherein the physical or logical array, or portion thereof or the master array or portion thereof, is heated or cooled by one or more of: a Peltier solid state heat pump, a heat pump, a resistive heater, a refrigeration unit, a heat sink, or a Joule Thompson cooling device.

**188.** The method of claim 161 or 169, further comprising producing a duplicate amplified physical or logical array of recombinant nucleic acids.

**189.** The method of claim 162 or 169, wherein screening the amplified physical or logical array of recombinant nucleic acids, or a duplicate thereof, for a desired property comprises: assaying a protein or product nucleic acid encoded by one or more members of the amplified physical or logical array of recombinant nucleic acids for one or more property.

**190.** The method of claim 161 or 169, further comprising in vitro transcribing members of the amplified physical or logical array of recombinant nucleic acids to produce an amplified array of in vitro transcribed nucleic acids.

**191.** The method of claim 128 or 169, comprising providing a first population of single-stranded template polynucleotides, which template polynucleotides are the same or different, and recombining the template polynucleotides by:

- (i) annealing a plurality of partially overlapping complementary nucleic acid fragments;  
and,  
(ii) extending the annealed fragments to produce a physical or logical array comprising a first population of recombinant nucleic acids.

**192.** The method of claim 191, comprising providing a physical array comprising the first population of template polynucleotides immobilized on a solid support.

**193.** The method of claim 192, wherein the solid support comprises a glass support, a plastic support, a silicon support, a chip, a bead, a pin, a filter, a membrane, a microtiter plate, or a slide.

**194.** The method of claim 192, wherein the first population of template polynucleotides comprises substantially an entire genome.

**195.** The method of claim 194, wherein the first population of template polynucleotides comprises a bacterial or fungal genome.

**196.** The method of claim 192, wherein the first population of template polynucleotides comprises substantially all of the expression products of a cell, tissue or organism.

**197.** The method of claim 196, wherein the first population of template polynucleotides comprises the expression products of a eukaryotic cell, tissue or organism.

**198.** The method of claim 192, wherein the first population of template polynucleotides comprises a subset of the expression products of a cell, tissue or organism.

**199.** The method of claim 198, wherein the first population of template polynucleotides comprises the expression products of a eukaryotic cell, tissue or organism.

200. The method of claim 192, the first population of template polynucleotides comprises a library of genomic nucleic acids or cellular expression products.

201. The method of claim 200, wherein the library of cellular expression products comprises a cDNA library.

202. The method of claim 191, wherein one or more template polynucleotides comprise one or more of a coding RNA, a coding DNA, an antisense RNA, and antisense DNA, a non-coding RNA, a non-coding DNA, an artificial RNA, an artificial DNA, a synthetic RNA, a synthetic DNA, a substituted RNA, a substituted DNA, a naturally occurring RNA, a naturally occurring DNA, a genomic RNA, a genomic DNA, or a cDNA.

203. The method of claim 161 or 169, further comprising in vitro transcribing members of the amplified physical or logical array of recombinant nucleic acids to produce an amplified array of transcribed nucleic acids and translating the amplified physical or logical array of transcribed nucleic acids to produce an amplified physical or logical array of polypeptides.

204. The method of claim 203, further comprising determining a concentration of polypeptide or transcribed nucleic acid at one or more positions in the amplified physical or logical array of polypeptides.

205. The method of claim 204, further comprising re-arraying the amplified physical or logical array of polypeptides or in vitro transcribed nucleic acids in a secondary polypeptide or in vitro transcribed nucleic acid array which has an approximately uniform concentration of polypeptides or in vitro transcribed nucleic acids at a plurality of locations in the secondary polypeptide array.

206. The method of claim 204, further comprising determining a correction factor which accounts for variation in polypeptide or in vitro transcribed nucleic acid concentrations at different positions in the amplified physical or logical array of polypeptides or in vitro transcribed nucleic acids.



207. The method of claim 203, further comprising adding one or more substrate to a plurality of members of the logical array of polypeptides or in vitro transcribed nucleic acids.

208. The method of claim 207, further comprising monitoring formation of a product produced by contact between the one or more substrate and one or more of the plurality of members of the logical array of polypeptides.

209. The method of claim 208, wherein the formation of the product is detected indirectly.

210. The method of claim 208, wherein the formation of the product is detected by a coupled enzymatic reaction which detects the product or the substrate or a secondary product of the product or substrate.

211. The method of claim 208, wherein the formation of the product is detected by monitoring peroxide production.

212. The method of claim 208, wherein the formation of the product is detected directly.

213. The method of claim 208, wherein the formation of the product is detected by monitoring production or heat or entropy which results from the formation of the product.

214. The method of claim 203, further comprising selecting the physical or logical array of polypeptides for a desired property, thereby identifying one or more selected member of the physical or logical array of polypeptides which has a desired property, thereby identifying one or more selected member of the amplified physical or logical array of recombinant nucleic acids that encodes the one or more member of the physical or logical array of polypeptides.

215. The method of claim 214, wherein selecting the physical or logical array is performed in a primary screening assay, the method further comprising one or more of:

- 09750040-014004
- (i) re-selecting the one or more selected member of the amplified physical or logical array of recombinant nucleic acids in a secondary screening assay;
  - (ii) quantifying protein levels at one or more location in the physical or logical array of polypeptides;
  - (iii) purifying proteins from one or more locations in the physical or logical array of polypeptides;
  - (iv) normalizing activity levels in the primary screen by compensating for protein quantitation at a plurality of locations in the physical or logical array of polypeptides;
  - (v) determining a physical characteristic of the one or more selected members; or,
  - (vi) determining an activity of the one or more selected members.

**216.** The method of claim 214, further comprising recombining the one or more selected member of the amplified physical or logical array of recombinant nucleic acids with one or more additional nucleic acids, *in vivo*, *in vitro* or *in silico*.

**217.** The method of claim 214, further comprising cloning or sequencing the one or more member of the amplified physical or logical array of recombinant nucleic acids.

**218.** The method of claim 161 or 169, further comprising selecting one or more member of the amplified physical or logical array, or a duplicate thereof, based upon the screening of the amplified physical or logical array for a desired property.

**219.** The method of claim 218, wherein a plurality of members of the amplified physical or logical array or duplicate thereof are selected, recombined and re-arrayed to form a secondary array of recombined selected nucleic acids, which secondary array is re-screened for the desired property, or for a second desired property.

**220.** A method of detecting or enriching for *in vitro* transcription or translation products, the method comprising:

localizing one or more first nucleic acids which encode one or more moieties proximal to one or more moiety recognition agents which specifically bind the one or more moieties;

in vitro translating or transcribing the one or more nucleic acids, thereby producing the one or more moieties, which one or more moieties diffuse or flow into contact with the one or more moiety recognition agents; and,

permitting binding of the one or more moieties to the one or more moiety recognition agents, and detecting or enriching for the one or more moieties by detecting or collecting one or more material proximal to, within or contiguous with the moiety recognition agent which material comprises at least one of the one or more moieties, which moieties individually comprise one or more in vitro translation or transcription product.

**221.** The method of claim 220, further comprising pooling the one or more moieties by pooling the material which is collected.

**222.** The method of claim 220, wherein the one or more moieties comprise one or more polypeptides or one or more RNAs.

**223.** The method of claim 220, wherein one or more moiety recognition agents comprise one or more antibody or one or more second nucleic acids.

**224.** The method of claim 220, wherein the first nucleic acids comprise a related population of shuffled nucleic acids.

**225.** The method of claim 220, wherein the first nucleic acids comprise a related population of shuffled nucleic acids, which shuffled nucleic acids encode an epitope tag, which epitope tag is bound by the moiety or the one or more moiety recognition agents.

**226.** The method of claim 220, wherein the first nucleic acids comprise a related population of shuffled nucleic acids and a PCR primer binding region, the method further comprising PCR amplifying a set of parental nucleic acids to produce the related population of shuffled nucleic acids.

**227.** The method of claim 220, wherein the first nucleic acids comprise a related population of shuffled nucleic acids and a PCR primer binding region, the

method further comprising identifying one or more target first nucleic acid by proximity to the moieties which are bound to the one or more moiety recognition agent, and amplifying the target first nucleic acid by hybridizing a PCR primer to the PCR primer binding region and extending the primer with a polymerase.

**228.** The method of claim 220, wherein the first nucleic acids comprise an inducible or constitutive heterologous promoter.

**229.** The method of claim 220, wherein the first nucleic acids and the one or more moiety recognition agents are localized on a solid substrate.

**230.** The solid substrate made by the method of claim 229.

**231.** The method of claim 229, wherein the solid substrate is a bead.

**232.** The method of claim 229, wherein the first nucleic acids and the one or more moiety recognition agents are localized on the solid substrate by one or more of: a cleavable linker chemical linker, an agarose gel, a colloid, a magnetic field, or an electrical field.

**233.** The method of claim 220, further comprising detecting an activity of the moiety or moiety recognition agent.

**234.** The method of claim 233, further comprising picking the one or more first nucleic acid with an automated robot.

**235.** The method of claim 233, further comprising picking the one or more first nucleic acid by placing a capillary on a region comprising the detected activity of the moiety or moiety recognition agent.

**236.** The method of claim 220, wherein the moiety or moiety in contact with the moiety recognition agent cleaves a cleavable linker, which linker attaches the first nucleic acid to a solid substrate.

**237.** A method of producing duplicate arrays of shuffled or mutagenized nucleic acids, the method comprising:

providing a physical or logical array of shuffled or mutagenized nucleic acids or transcribed shuffled or transcribed mutagenized nucleic acids; and,

forming a duplicate array of copies of the shuffled or mutagenized nucleic acids or copies of the transcribed shuffled or transcribed mutagenized nucleic acids by physically or logically organizing the copies into a physical or logical array.

**238.** The physical or logical array and duplicate array produced by the method of claim 237.

**239.** The method of claim 237, wherein the copies are produced by copying the shuffled or mutagenized nucleic acids or transcribed shuffled or transcribed mutagenized nucleic acids using a polymerase or an in vitro nucleic acid synthesizer.

**240.** The method of claim 237, further comprising forming an array of reaction mixtures which corresponds to the physical or logical array of shuffled or mutagenized nucleic acids or transcribed shuffled or transcribed mutagenized nucleic acids, which reaction mixtures comprise members of the array of shuffled or mutagenized nucleic acids or transcribed shuffled or transcribed mutagenized nucleic acids or the duplicate array of copies of the shuffled or mutagenized nucleic acids or copies of the transcribed shuffled or transcribed mutagenized nucleic acids, or a derivative copy thereof.

**241.** The method of claim 240, wherein the reaction mixtures further comprise one or more in vitro transcription or translation reagent.

**242.** A method of normalizing an array of reaction mixtures, the method comprising:

in vitro transcribing or translating a physical or logical array of shuffled or mutagenized nucleic acids or transcribed shuffled or transcribed mutagenized nucleic acids to produce an array of products; and,

determining a correction factor which accounts for variation in concentration of the products at different sites in the array of products.

**243.** The method of claim 242, further comprising producing a secondary product array, which secondary array comprises selected concentrations of the products at one or more sites in the secondary array.

**244.** The physical or logical array of shuffled or mutagenized nucleic acids or transcribed shuffled or transcribed mutagenized nucleic acids, the array of products and the secondary array produced by the method of claim 243.

**245.** The method of claim 243, wherein the secondary array is formed by transferring an aliquot from a plurality of sites in the array of products to a plurality of secondary sites in the secondary array.

**246.** The method of claim 245, further comprising diluting the products during said transferring or after transfer to the secondary sites, thereby selecting the concentration of the products at the secondary sites in the secondary array.

**247.** A method for recombining one or more nucleic acids, the method comprising:

- (a) immobilizing one or more template nucleic acids on a solid support;
- (b) annealing a plurality of partially overlapping complementary nucleic acid fragments to the immobilized template nucleic acid;
- (c) extending or ligating the annealed fragments to produce at least one heteroduplex, which heteroduplex comprises a template nucleic acid and a substantially full-length heterolog complementary to the template nucleic acid; and,
- (d) recovering at least one substantially full-length heterolog.

**248.** The method of claim 247, comprising immobilizing a plurality of template nucleic acids on a solid support.

**249.** The method of claim 248, wherein the plurality of template nucleic acids comprises substantially an entire genome.

**250.** The method of claim 249, wherein the plurality of template nucleic acids comprises a bacterial or fungal genome.

**251.** The method of claim 248, wherein the plurality of template nucleic acids comprises substantially all of the expression products of a cell, tissue or organism.

**252.** The method of claim 251, wherein the plurality of template nucleic acids comprises the expression products of a eukaryotic cell, tissue or organism.

**253.** The method of claim 248, wherein the plurality of template nucleic acids comprises a subset of the expression products of a cell, tissue or organism.

**254.** The method of claim 253, wherein the plurality of template nucleic acids comprises the expression products of a eukaryotic cell, tissue or organism.

**255.** The method of claim 248, wherein the plurality of template nucleic acids comprises a library of genomic nucleic acids or cellular expression products.

**256.** The method of claim 255, wherein the library of cellular expression products comprises a cDNA library.

**257.** The method of claim 248, comprising immobilizing the plurality of template nucleic acids in a spatial array.

**258.** The method of claim 247, wherein the one or more template nucleic acids comprise one or more of: a DNA, an RNA, a coding RNA, a coding DNA, an antisense RNA, an antisense DNA, a non-coding RNA, a non-coding DNA, an artificial RNA, an artificial DNA, a synthetic RNA, a synthetic DNA, a substituted RNA, a substituted DNA, a naturally occurring RNA, a naturally occurring DNA, a genomic RNA, a genomic DNA, or a cDNA.

**259.** The method of claim 247, comprising immobilizing one or more template nucleic acids on a solid support selected from among a glass support, a plastic support, a silicon support, a chip, a bead, a pin, a filter, a membrane, a microtiter plate, and a slide.

260. The method of claim 247, comprising immobilizing the one or more template nucleic acids by depositing a solution comprising the one or more template nucleic acids on a glass slide, which glass slide is coated with a polycationic polymer.

261. The method of claim 260, wherein the polycationic polymer comprises polylysine or polyarginine.

262. The method of claim 259, comprising immobilizing the one or more template nucleic acids by tethering the one or more template nucleic acids to the solid support.

263. The method of claim 262, wherein tethering comprises chemical tethering, biotin-mediated binding, uv cross-linking, fluorescence activated cross-linking, or heat mediated cross-linking.

264. The method of claim 247, comprising enzymatically extending the annealed fragments with a DNA or RNA polymerase.

265. The method of claim 264, comprising enzymatically extending the annealed fragments with a thermostable polymerase.

266. The method of claim 247, comprising enzymatically extending the annealed fragments with a ligase or nuclease, which ligase or nuclease comprises polymerase activity.

267. The method of claim 247, comprising extending and ligating the annealed fragments to produce at least one substantially full-length heterolog. A substantially full-length heterolog produced by the method of claim 247.

268. An array comprising a plurality of heteroduplexes or full-length heterologs produced by the method of claim 247.

269. The method of claim 247, comprising recovering the at least one substantially full-length heterolog by

(i) denaturing the heteroduplex;



- (ii) annealing at least one oligonucleotide primer to the heterolog; and,  
(iii) extending the oligonucleotide primer to produce a duplex polynucleotide.

**270.** The method of claim 269, further comprising amplifying the duplex polynucleotide.

**271.** The method of claim 270, comprising amplifying the duplex polynucleotide using a boomerang sequence, a splinkerette or a vectorette.

**272.** An amplified heterolog produced by the method of claim 270.

**273.** The method of claim 269, further comprising introducing the duplex polynucleotide into a cell.

**274.** The method of claim 273, comprising introducing the duplex polynucleotide into a cell via a vector.

**275.** The method of claim 274, wherein the vector is a plasmid, a cosmid, a phage or a transposon.

**276.** A vector produced by the method of claim 274.

**277.** A cell produced by the method of claim 273.

**278.** The method of claim 247, further comprising identifying at least one substantially full-length heterolog with a desired property.

**279.** The method of claim 278, comprising identifying the at least one substantially full-length heterolog with a desired property in an automated or partially automated high-throughput assay system.

**280.** The method of claim 247, further comprising:

- (i) recombining or mutating the at least one substantially full-length heterolog to produce a library of diversified heterologs; and  
(ii) optionally, identifying at least one diversified heterolog with a desired property.

280. **281.** A library of diversified heterologs produced by the method of claim

**282.** An integrated system comprising an array, which array comprises a plurality of heteroduplexes or full-length heterologs produced by the method of claim 247.

**283.** The integrated system of claim 282, further comprising one or more of a detector, a data input device, a data output device, a data storage device, and a controller.

**284.** The integrated system of claim 283, wherein the controller comprises one or more of a fluid handling mechanism, an array mobilization mechanism, and an array storage device.

**285.** A method of directing nucleic acid fragmentation using a computer, the method comprising: calculating a ratio of uracil to thymidine, which ratio when used in a fragmentation module produces one or more nucleic acid fragment of a selected length.

**286.** A method of directing PCR using a computer, the method comprising: calculating one or more crossover region between two or more parental nucleic acid sequence using one or more annealing temperature or extension temperature.

**287.** The method of claim **286**, comprising calculating the one or more crossover region using one or more theoretical prediction or one or more set of empirical data to calculate a melting temperature.

**288.** A method of selecting one or more parental nucleic acids for diversity generation using a computer, the method comprising:

- (i) performing an alignment between two or more potential parental nucleic acid sequences;
- (ii) calculating a number of mismatches between the alignment;

- 09760040-014004
- (iii) calculating a melting temperature for one or more window of  $w$  bases in the alignment;
  - (iv) identifying one or more window of  $w$  bases having a melting temperature greater than  $x$ ;
  - (v) identifying one or more crossover segment in the alignment, which one or more crossover segment comprises two or more windows having a melting temperature greater than  $x$ , which two or more windows are separated by no more than  $n$  nucleotides;
  - (vi) calculating a dispersion of the one or more crossover segments;
  - (vii) calculating a first score for each alignment based on the number of windows having a melting temperature greater than  $x$ , the dispersion, and the number of crossover segments identified;
  - (viii) calculating a second score based on the number of mismatches, the number of windows having a melting temperature greater than  $x$ , the dispersion, and the number of crossover segments identified; and,
  - (ix) selecting one or more parental nucleic acid based on the first score and/or the second score.

**289.** The method of claim **288**, further comprising repeating steps (i) through (viii) starting with the one or more parental nucleic acid selected in step (ix).

**290.** The method of claim **288**, further comprising repeating steps (i) through (viii) starting with the one or more potential parental nucleic acid sequences and one or more different input parameters for calculating the melting temperature in step (ii).

**291.** The method of claim **288**, wherein the alignment comprises a pairwise alignment.

**292.** The method of claim **288**, wherein  $w$  comprises an odd number.

**293.** The method of claim **288**, wherein  $w$  comprises about 21.

294. The method of claim 288, further comprising calculating the melting temperature for the one or more window of w bases in the alignment from one or more set of empirical data or one or more melting temperature prediction algorithm.

295. The method of claim 288, wherein x is about 65 °C.

296. The method of claim 288, wherein n is about 2.

297. The method of claim 288, wherein the dispersion comprises the inverse of the average number of bases between crossover segments in the alignment.

298. The method of claim 288, wherein the instruction set selects the two or more potential parental nucleic acid sequences by searching one or more database for one or more nucleic acid sequence of interest and one or more homolog of the one or more nucleic acid sequence of interest.

299. A web page for directing nucleic acid diversity generation, the web page comprising a computer readable medium that causes a computer to perform the method of claim 285, claim 286, or claim 288.

09750070-011001